

*Molecular Biology Consortium*

## **Pixels and Proteins: Detectors for Protein Crystallography**

***Edwin M. Westbrook, John Morse,  
Sherwood Parker, Chris Kenney***

***Molecular Biology Consortium Inc.***



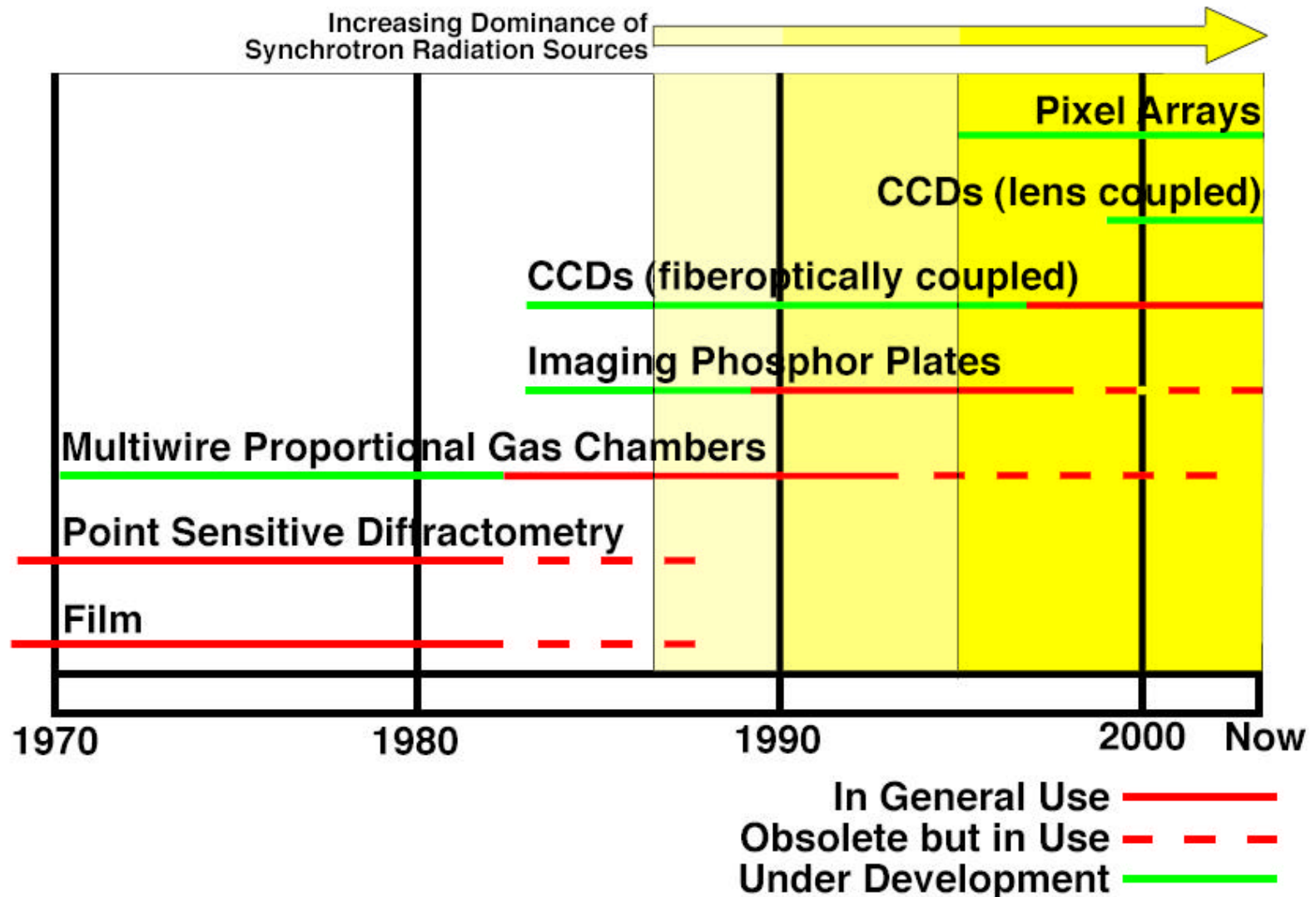
# Collaborations and Acknowledgements



- At Berkeley: Emanuelle Mandelli, Gerrit Meddeler,
  - Many others
  
- Supported by:
  - NIH grant R01 RR16334
  - NIH grant R01 RR16230
  - The Molecular Biology Consortium Inc.



# Historical Perspective of Protein Crystallographic Detectors

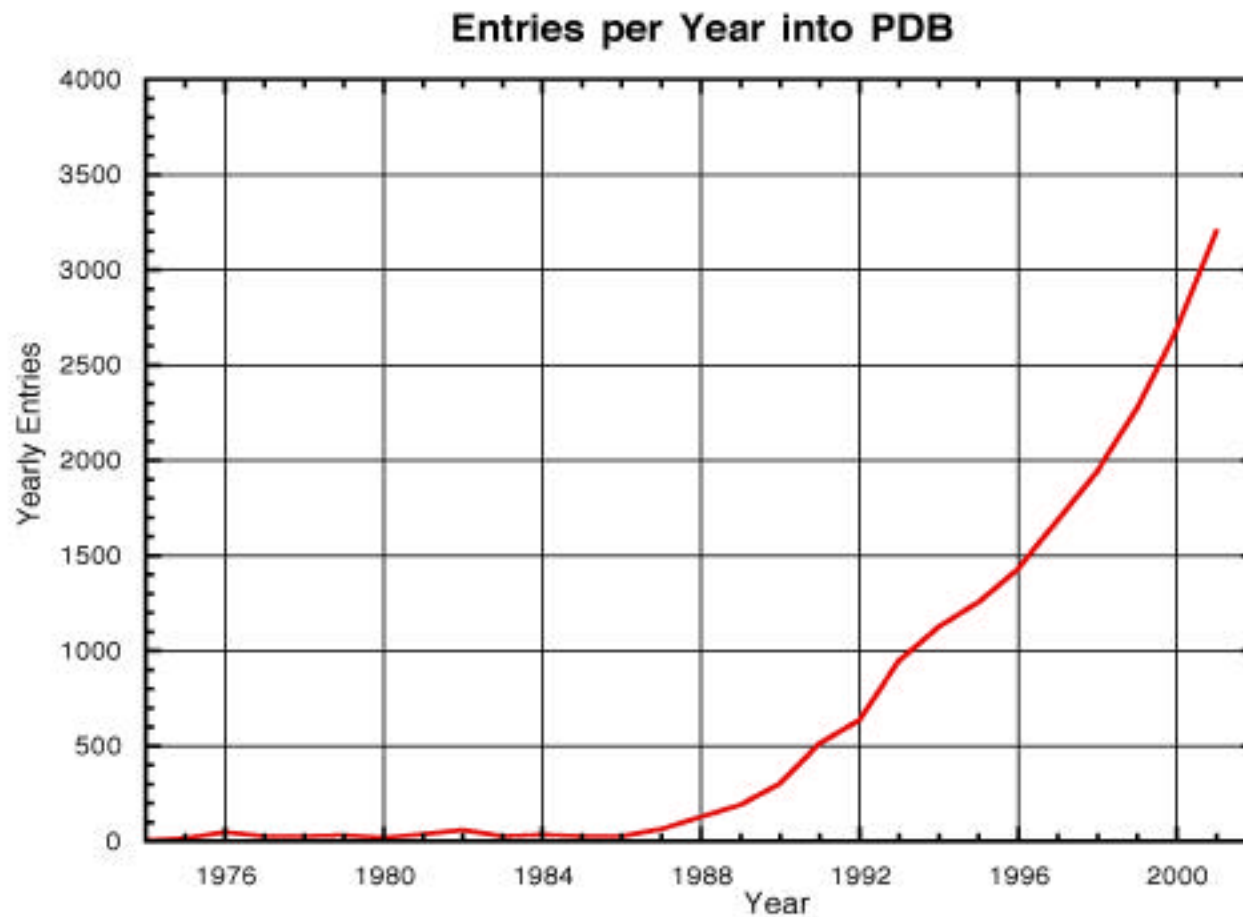




# Entries into the Protein Data Bank are increasing exponentially



There are now more than 20,000 entries in the PDB.





# Quality Parameters For Protein Crystallographic X-ray Detectors



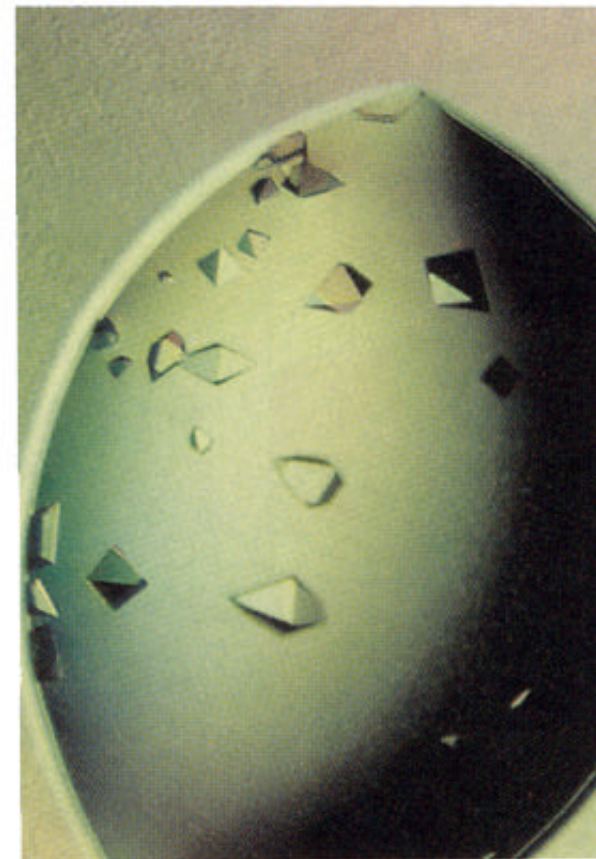
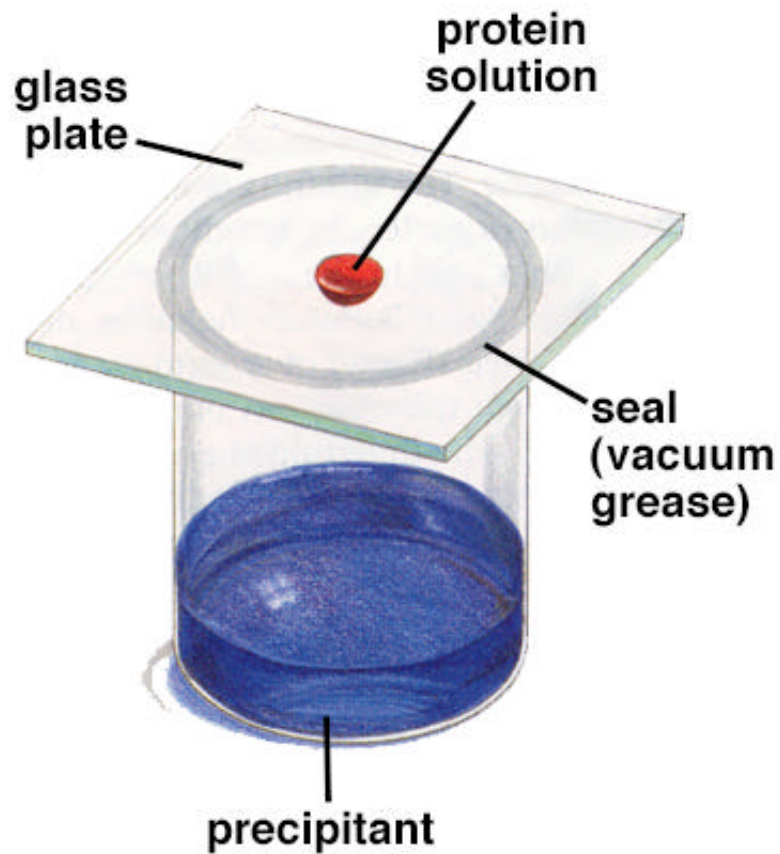
(A) Dynamic Range  
Noise  
Efficiency  
Sensitivity

(B) Speed: driven by crystal sample decay

(C) Resolving Power =  
(# Bragg orders across face)  
Physical Size



Protein Crystals are (almost)  
always grown from vapor  
diffusion droplets.

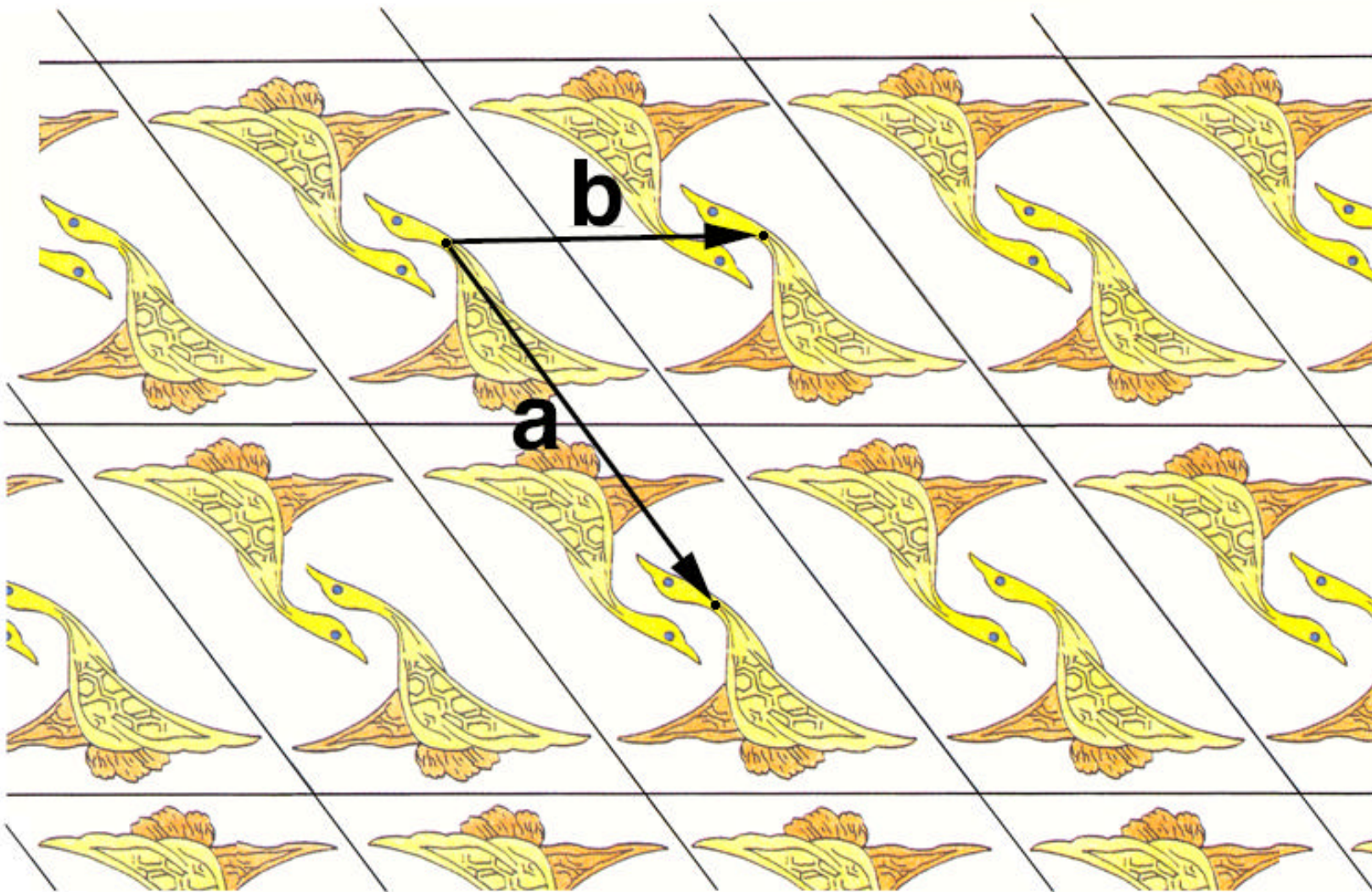




Crystals are symmetric w.r.t.  
translation operations:

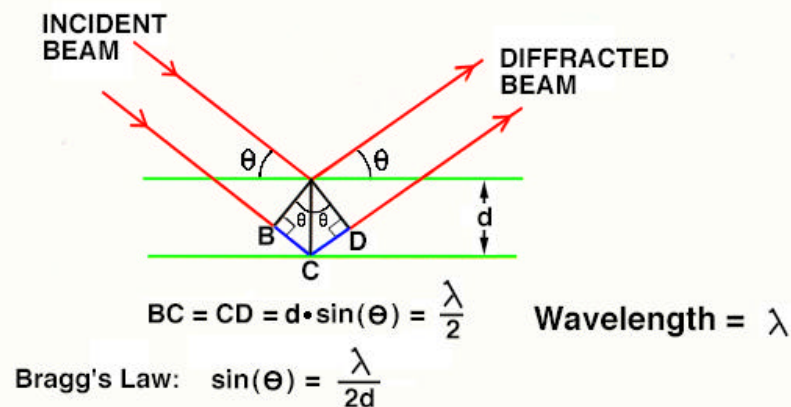
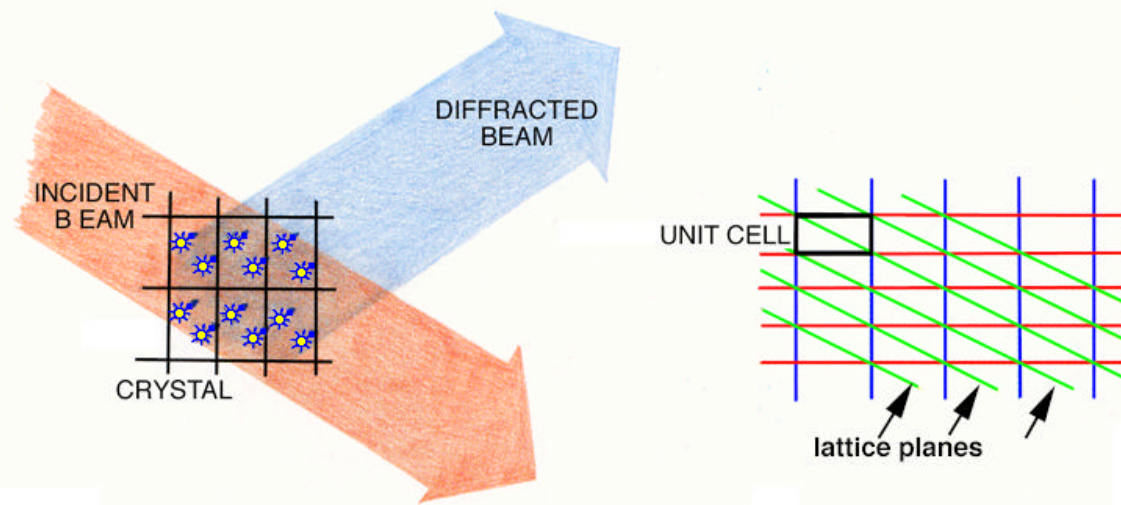
$$(x',y',z') = (x,y,z) + (na,mb,lc)$$

$a,b,c$  vectors,  $n,m,l$  integers



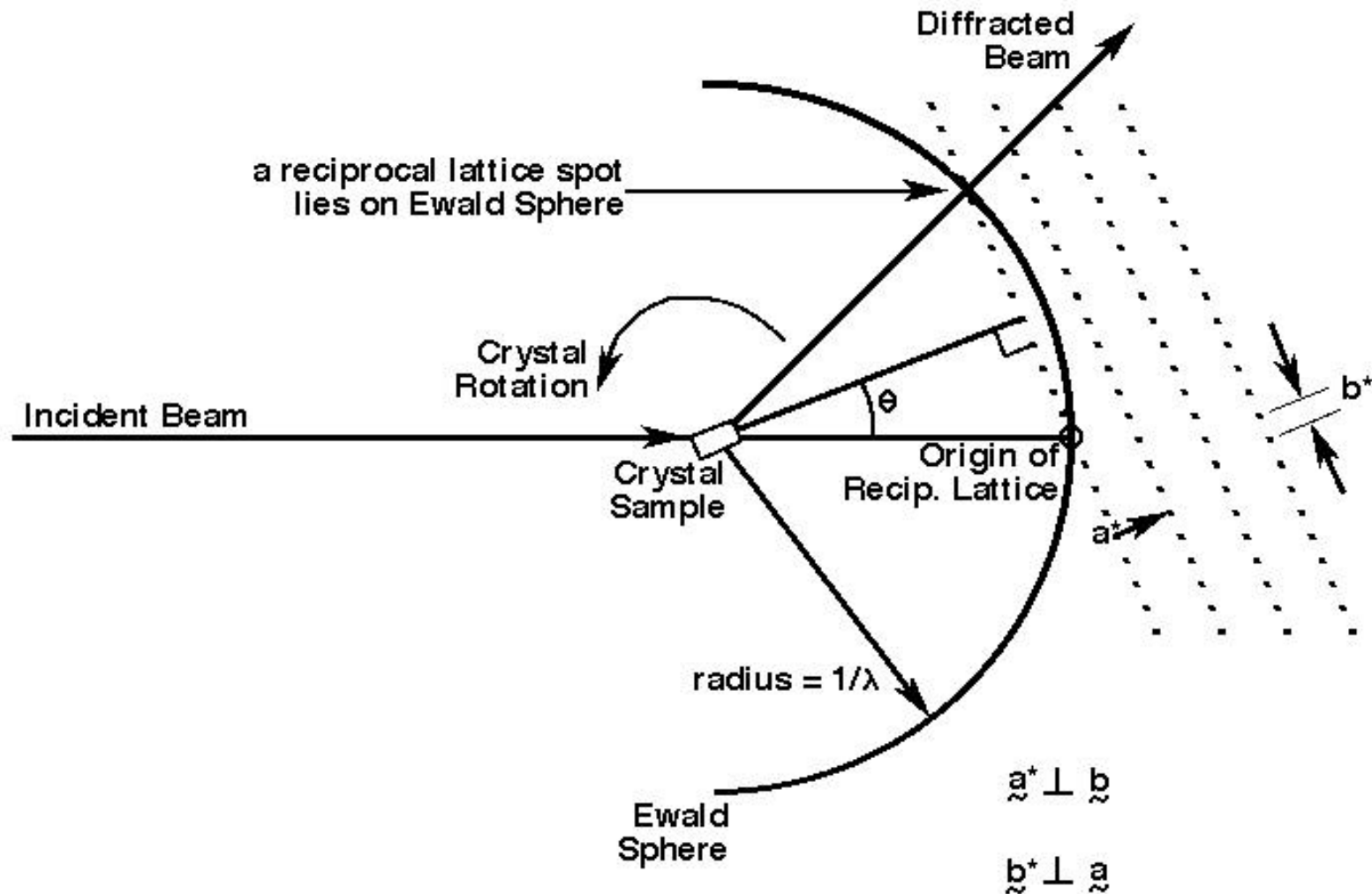


# Crystal Diffraction Occurs only at discrete momentum transfer directions: the Bragg Reflections.



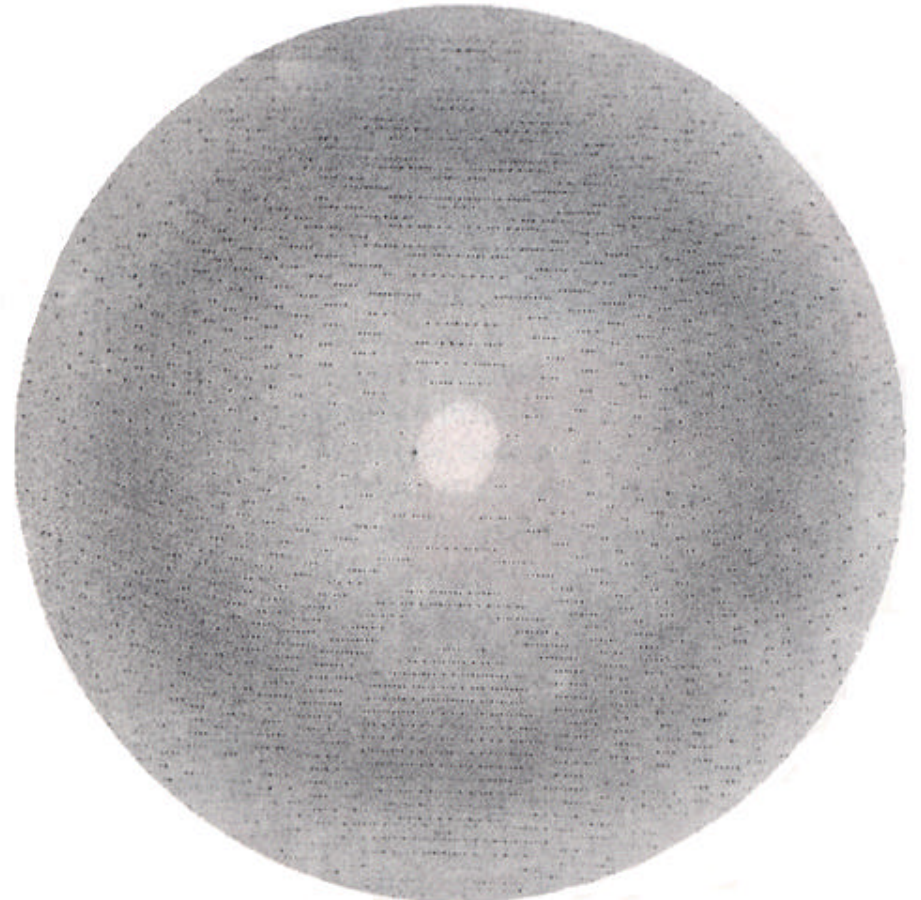
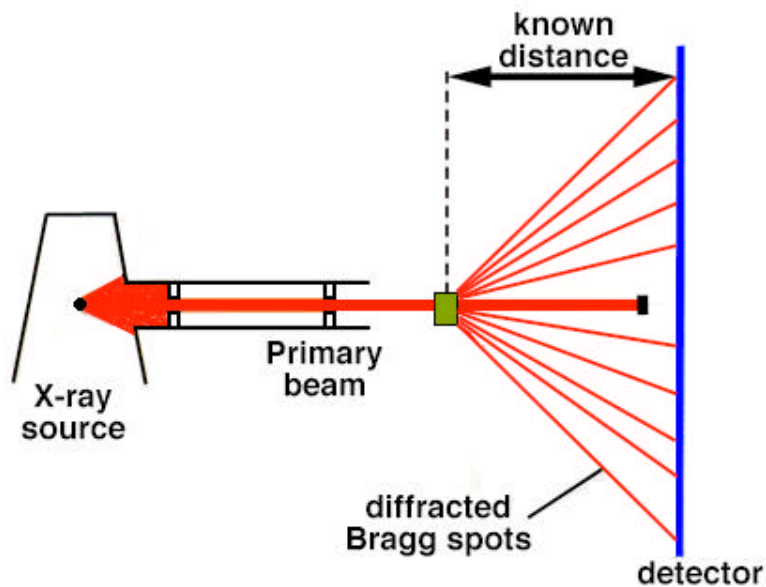


Each Bragg spot diffracts only at its Bragg angle. The crystal must be rotated to record all Bragg spot intensities.

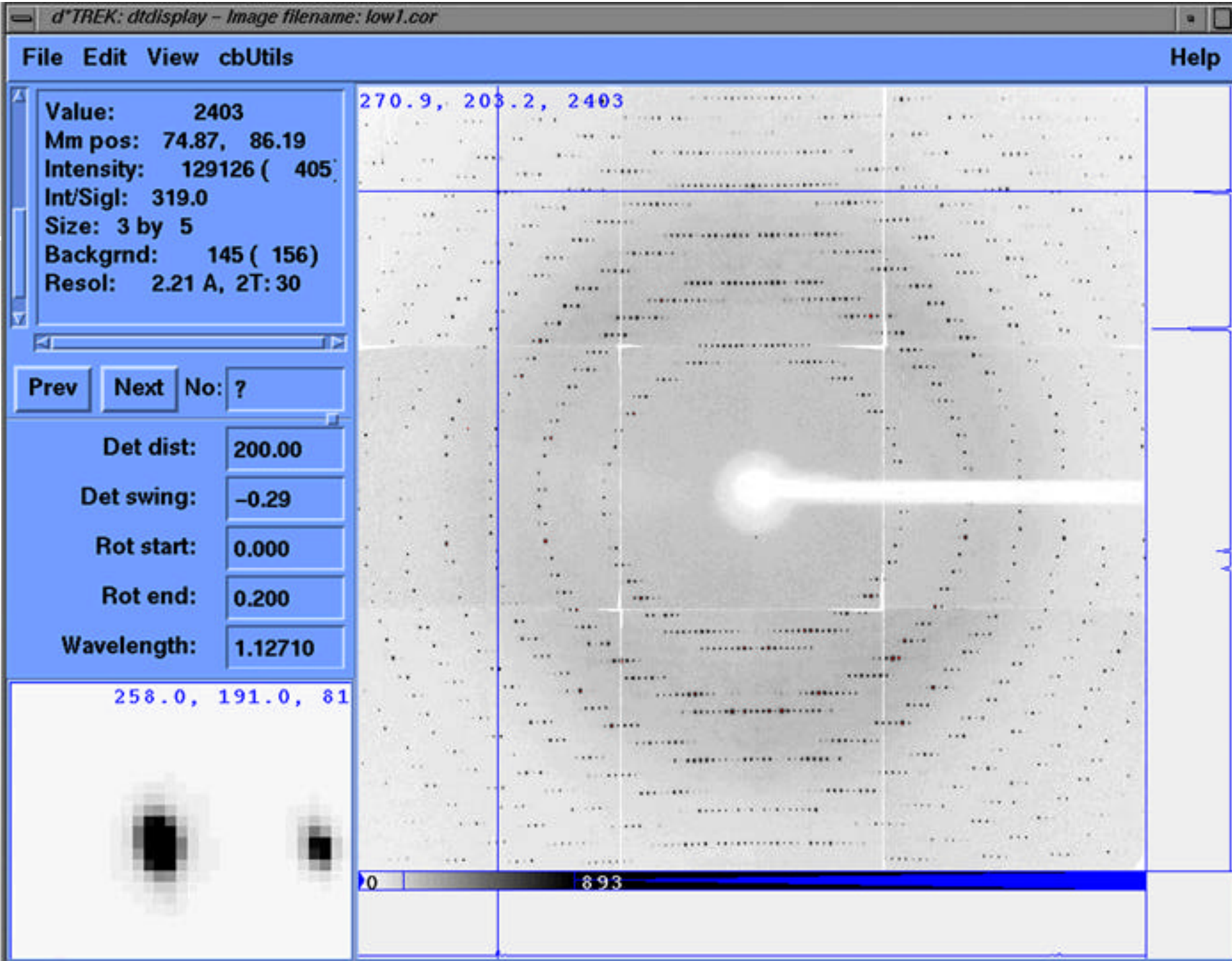




# The Diffraction Pattern of Discrete Bragg Spots is Captured by the Detector

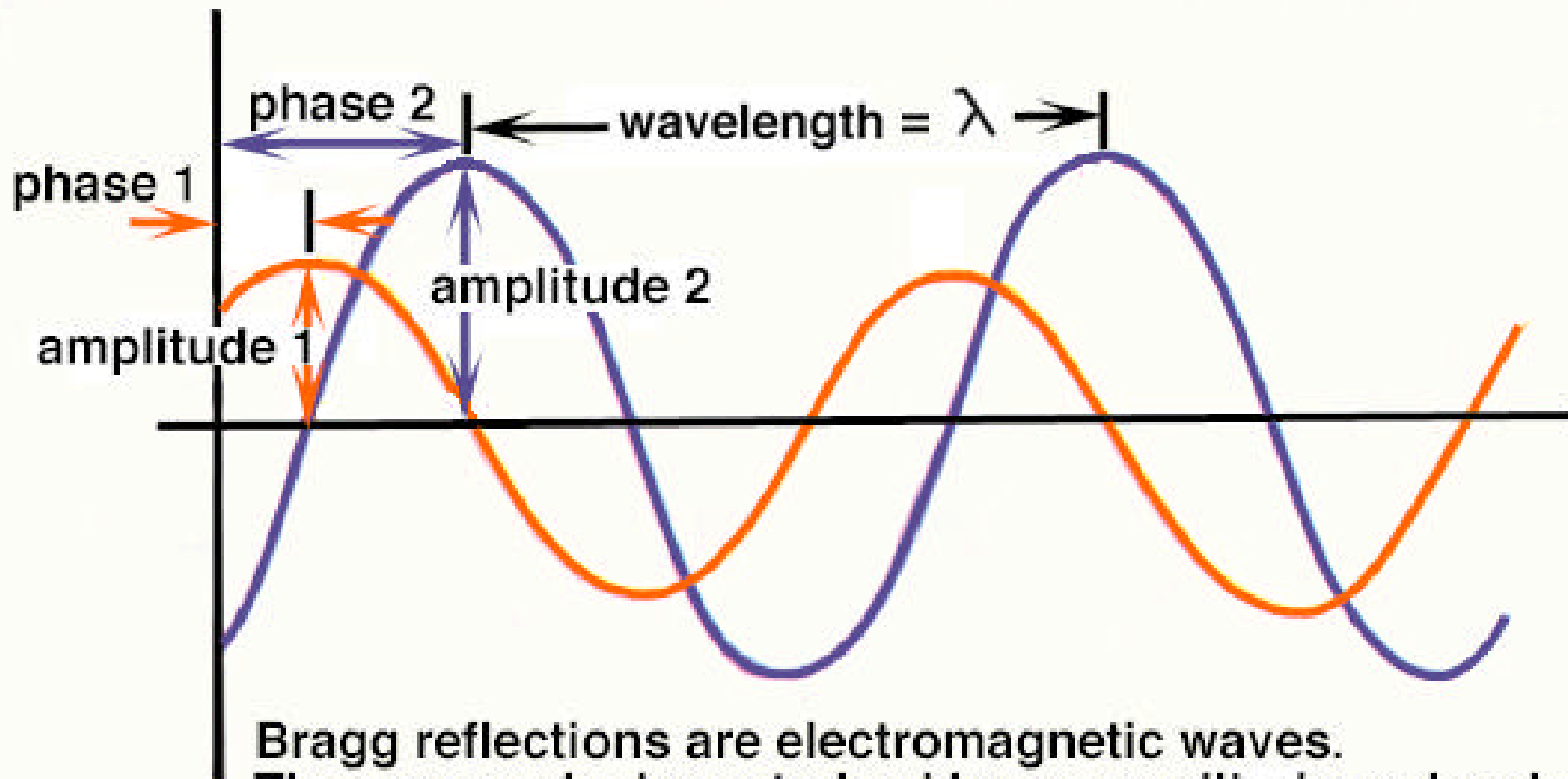








You can measure amplitude, but phases must be derived indirectly.



Bragg reflections are electromagnetic waves.  
They are each characterized by an amplitude and a phase.

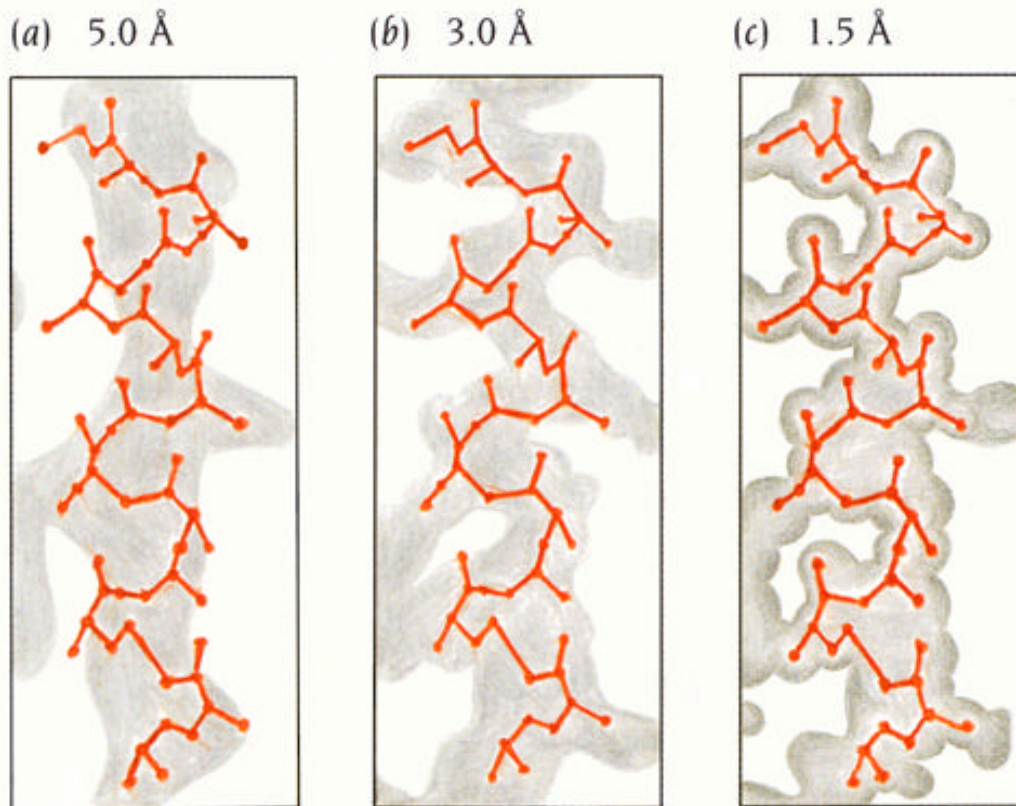
$$F(h,k,l) = A(h,k,l) e^{i\theta(h,k,l)}$$



Once phases are known, the electron density map can be calculated by a Fourier transform.



$$\rho(x, y, z) = \sum_{h \quad k \quad l} |F(h, k, l)| e^{i\phi(h, k, l)} e^{-2\pi i(hx + ky + lz)}$$



The molecular structure must be inferred from the map by someone competent in physical biochemistry.

The precision of the map interpretation depends on the resolution of the data to which you collected your data.



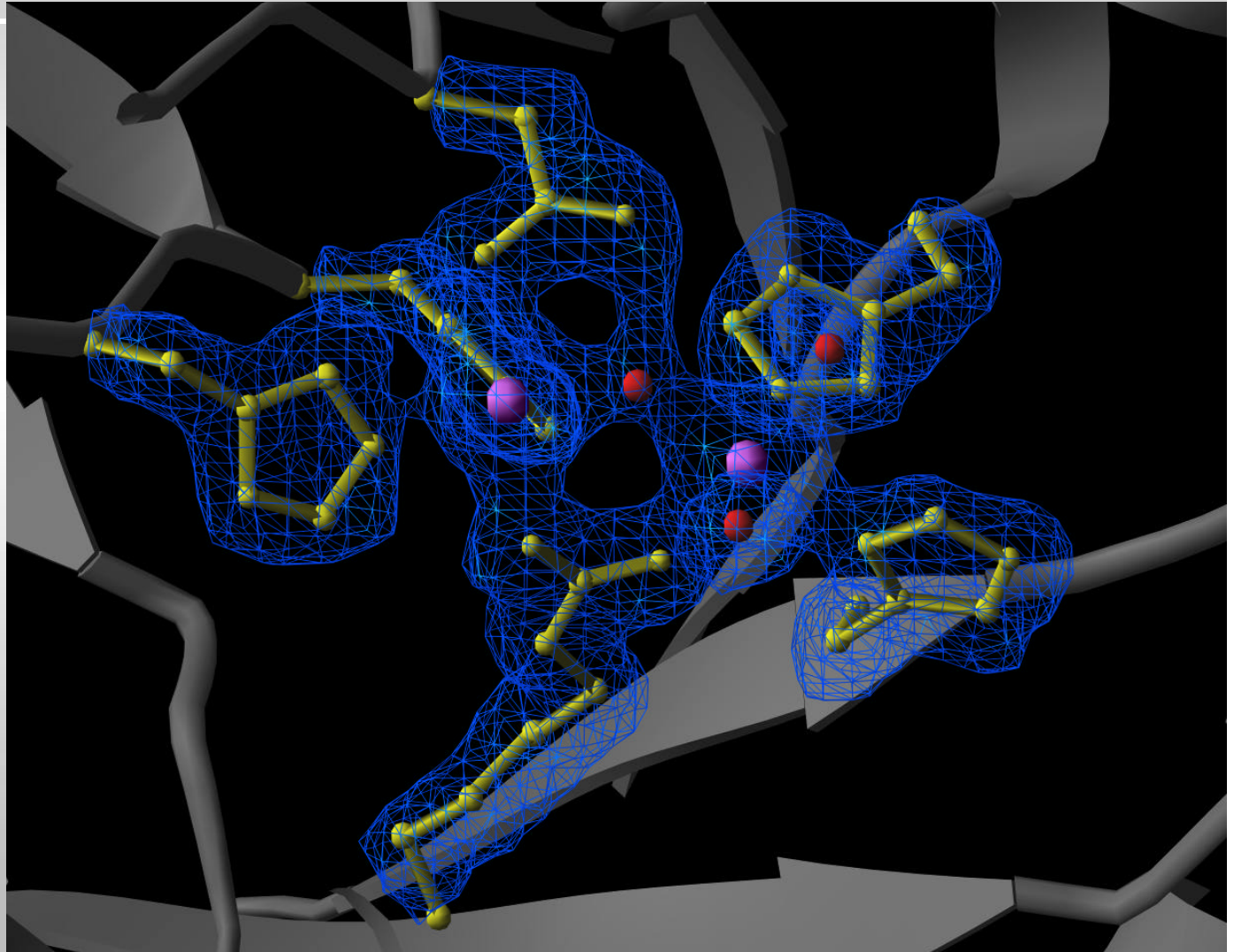
# Example: Structure of an Enzyme Active Site



■ **Pseudomonas  
diminuta  
phosphotriesterase:**

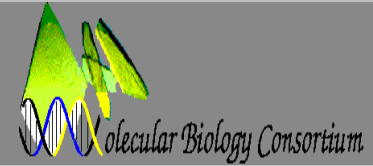
This enzyme catalyzes the hydrolysis of organophosphorus pesticides and nerve agents. Its crystal structure is being studied by Hazel Holden's research group at the University of Wisconsin, Madison (see PDB file 1DPM).

- Purple atoms: zinc
- Red: bound water
- Yellow: side chains
- 1.8 Å resolution map,
- 21% R-factor



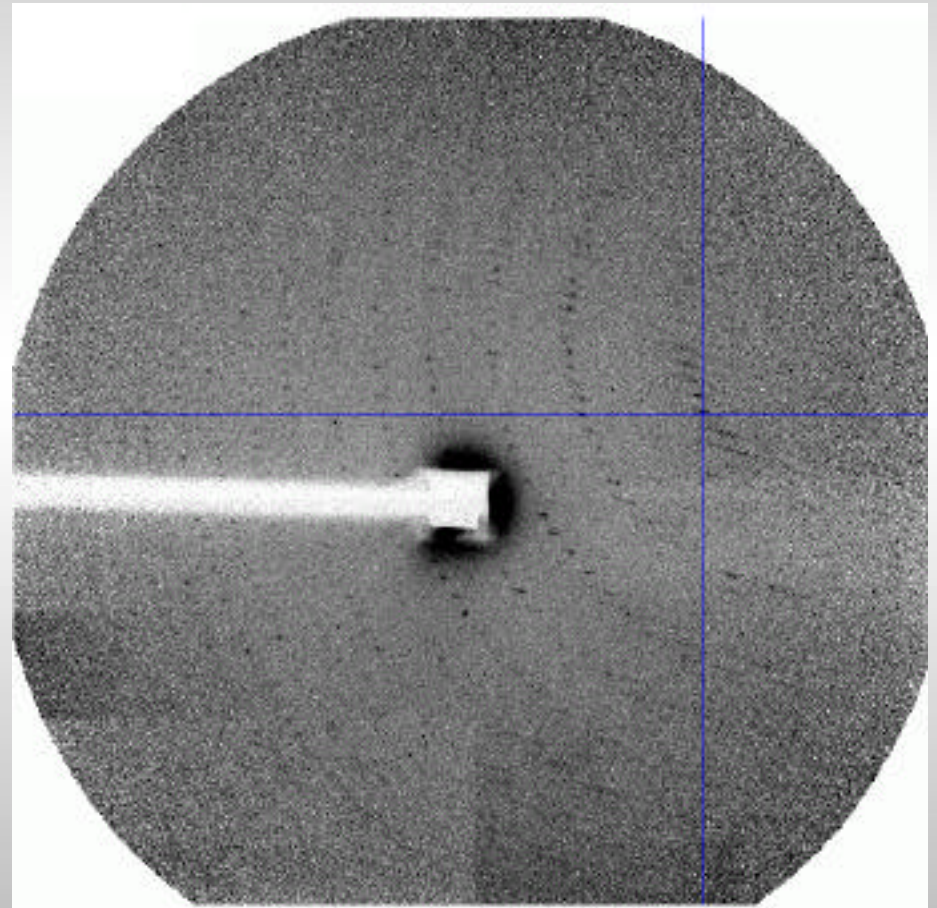
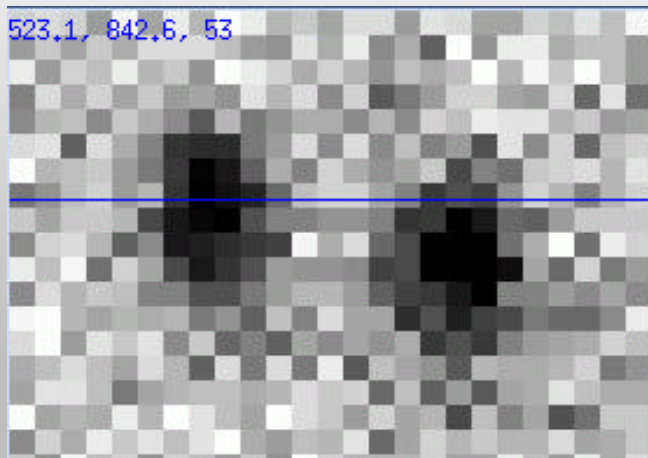


# Virus Crystal Diffraction: 600 Å Unit Cell



- Data taken at SBCCAT bending magnet line, 19BM, at APS.
- Image of virus diffraction pattern, detector distance = 550mm, Wavelength  $\lambda = 1.5 \text{ Å}$ .

Data to edge of detector, 5.8 Å.  
Below - pixel blowup showing separation of 600 Å axis spots.



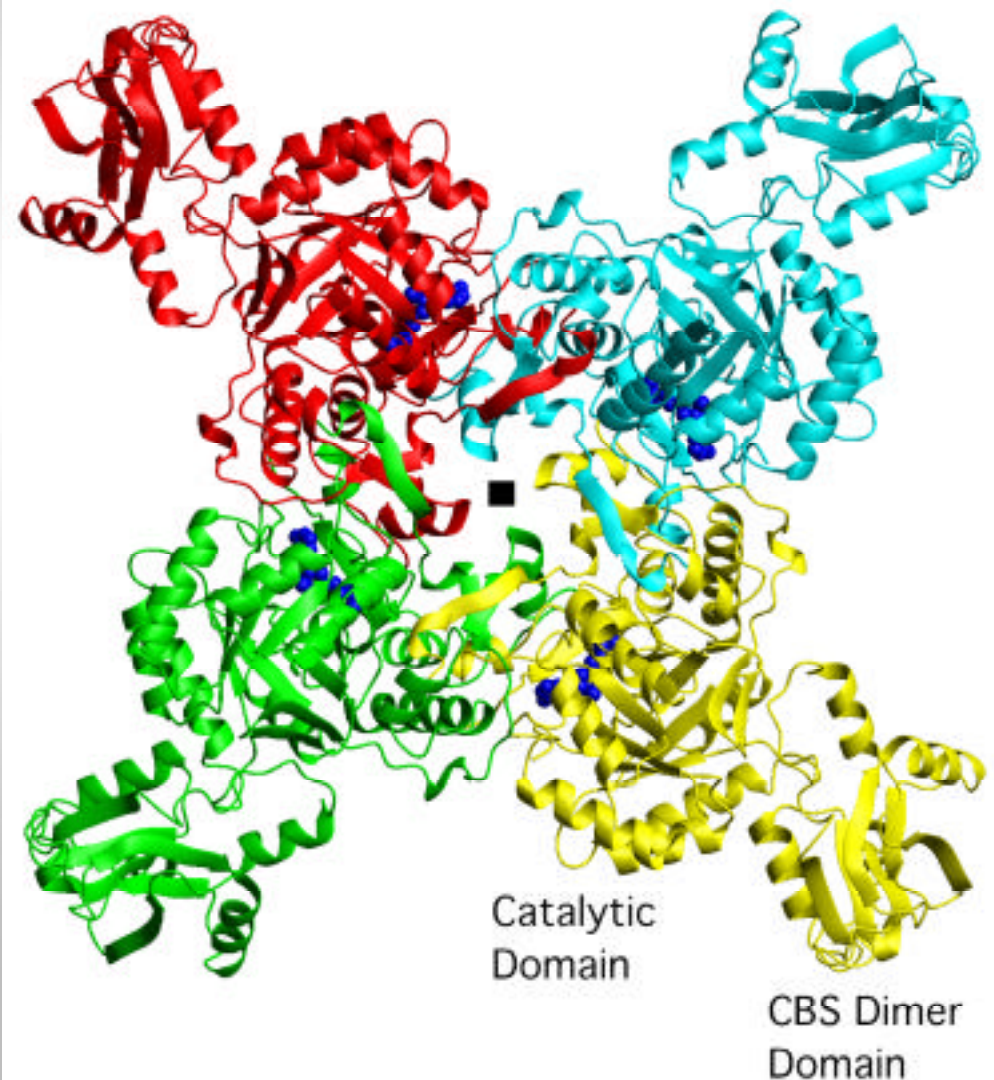
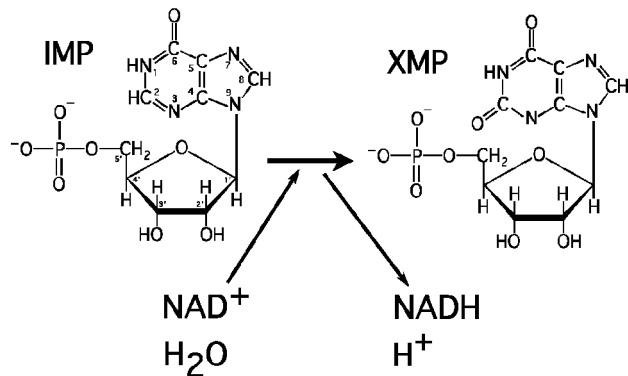
- Thanks to Michael Chapman, Florida State University



# IMPDH Story: Compare Bacterial (Staphylococcus) Enzyme with Human: drug design



## Structure and Function of Inosine Monophosphate Dehydrogenase





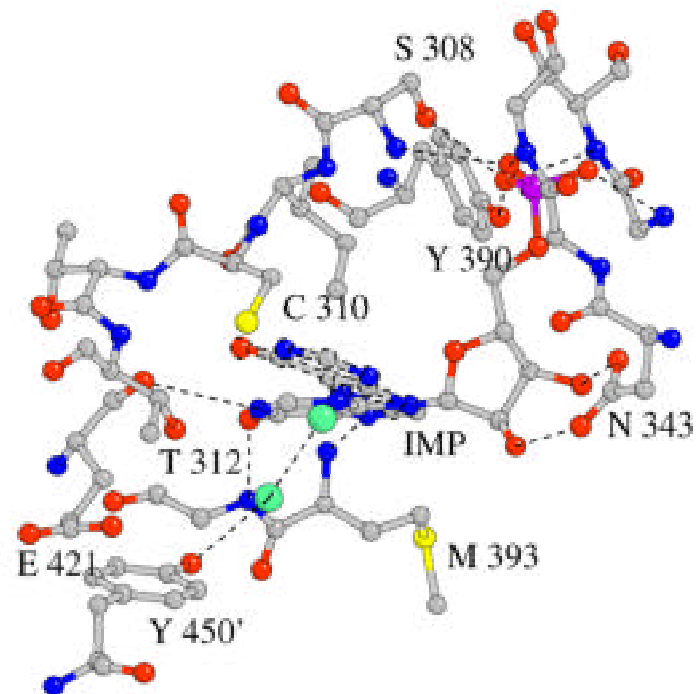
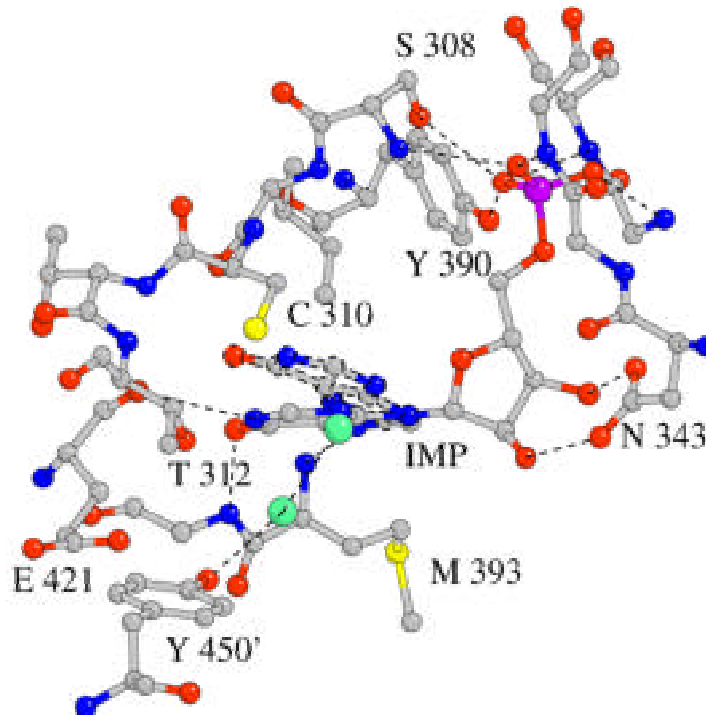
## S. pyogenes IMPDH: Crystal Parameters and Data Summaries (data from fiber-optic/CCD detector)



- Unit cell:      Space group: I422
- $a = b = 151.5 \text{ \AA}$ ,  $c = 101.7 \text{ \AA}$
- High Resolution data set (one crystal):
  - Resolution:  $10 \text{ \AA} - 1.9 \text{ \AA}$
  - Wavelength:  $1.0332 \text{ \AA}$
  - Exposure times: 8 seconds/degree of rotation
  - # Bragg spots observed: 263,355
  - # Unique reflections: 44,921
  - Redundancy: 5.9-fold      100 data images
  - Completeness: 96.5%      20 minutes to
  - $R_{\text{merge}}$  6.8%      record all data

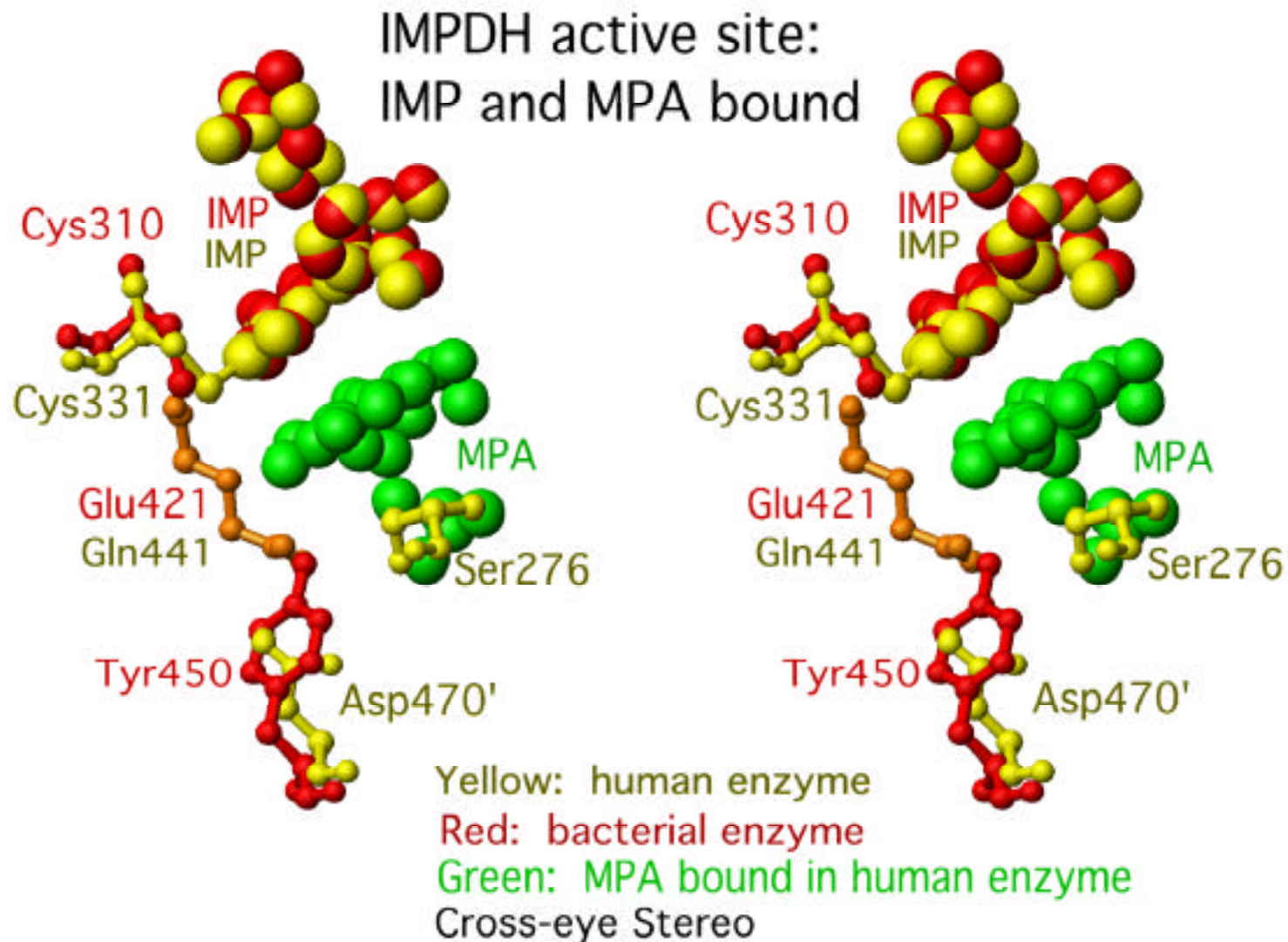


# Molecular Model of Bacterial IMPDH Enzyme Active Site



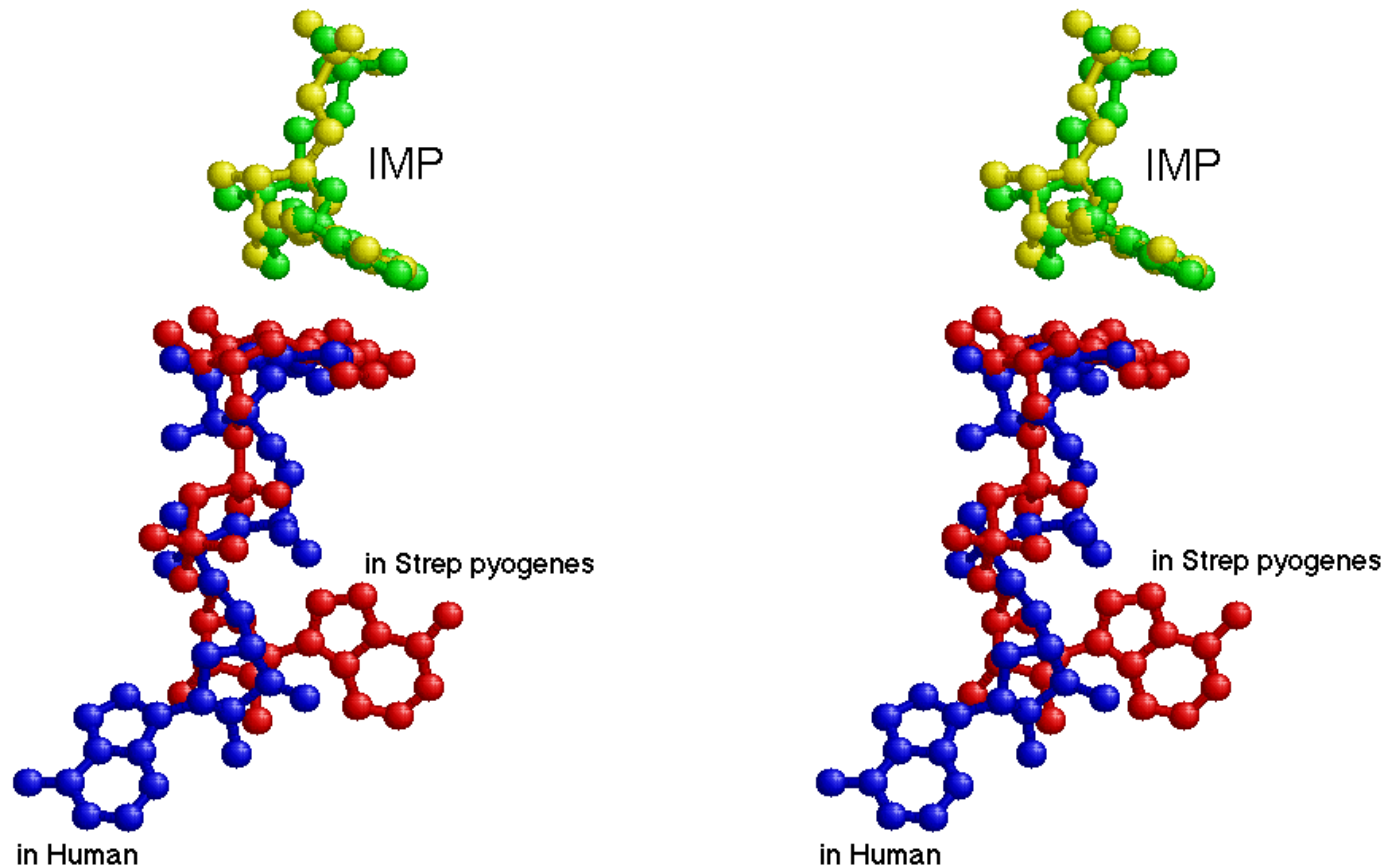


# Correspondence between Bacterial and Human IMPDH Active Sites: IMP/MPA binding





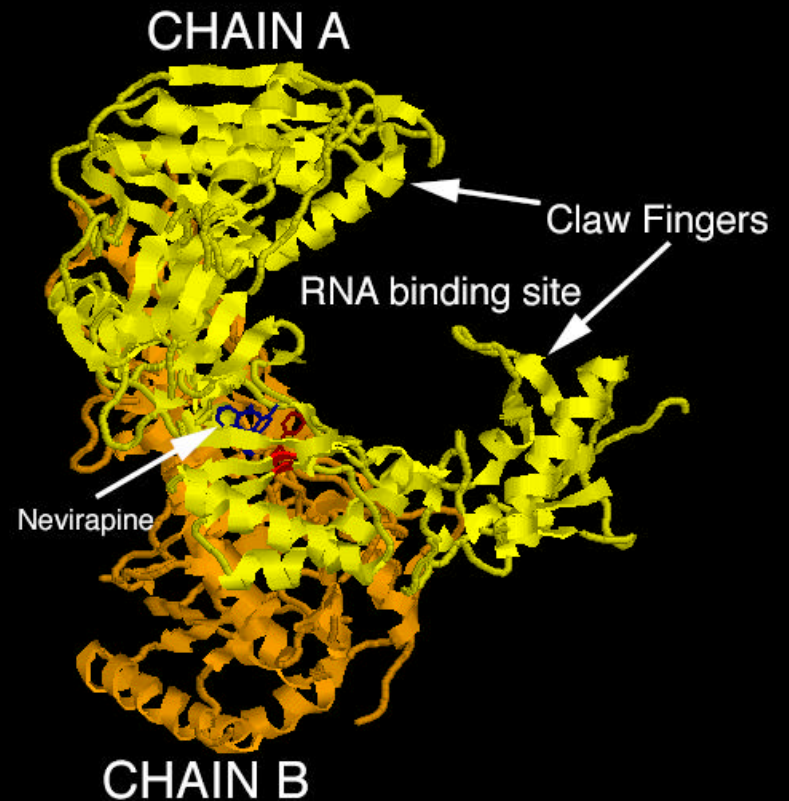
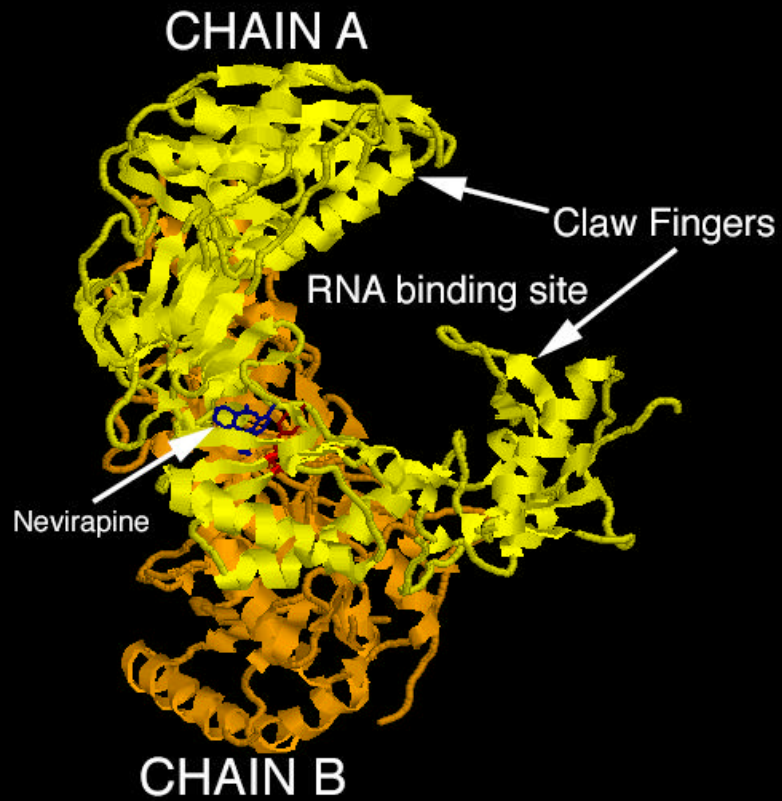
# A Striking Difference Between Human and Bacterial Binding of NAD Analog TAD



Alternate sites of Adenine in 2 crystal structures

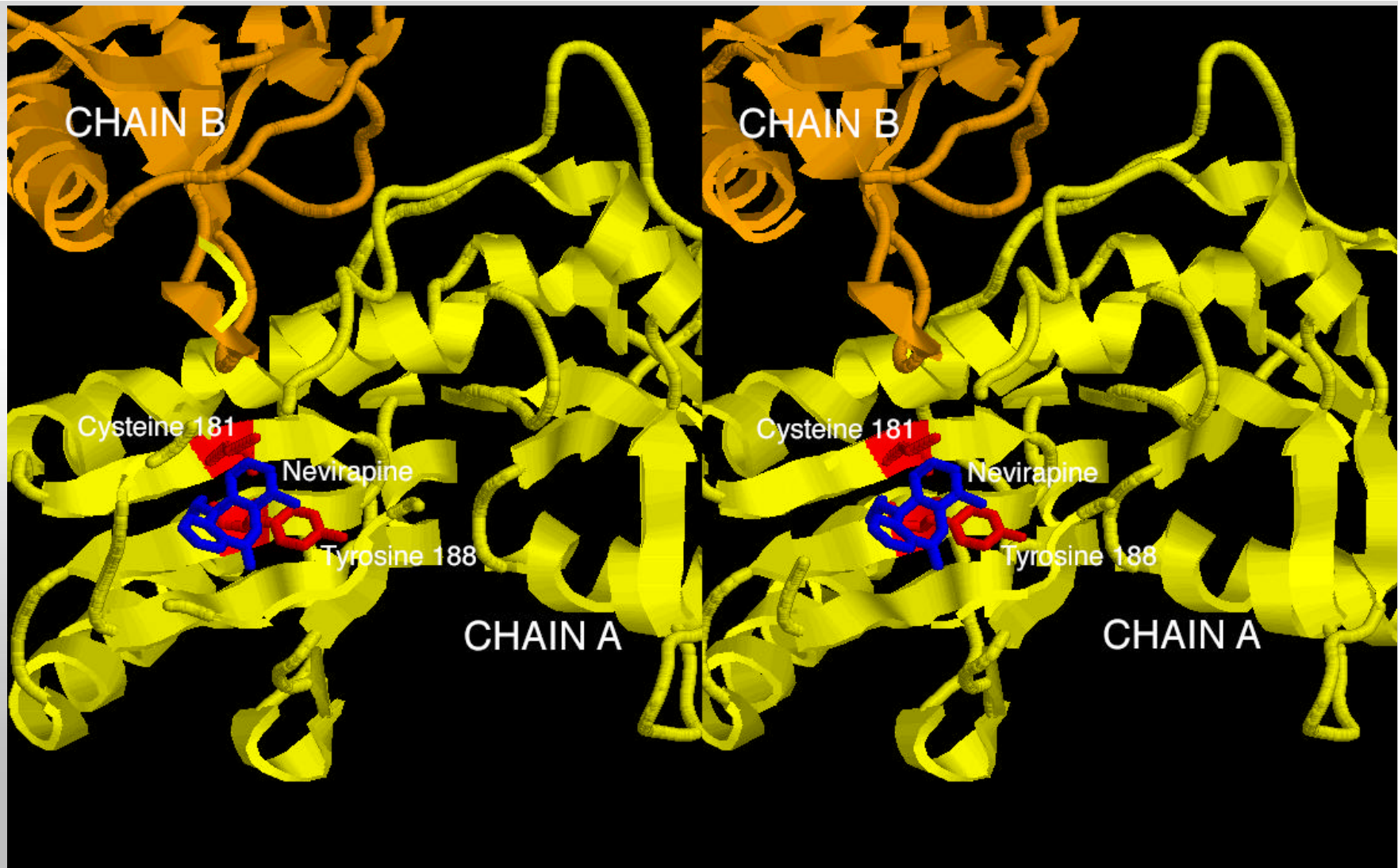
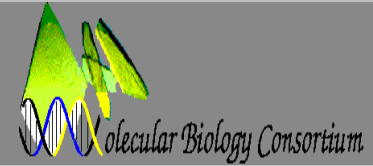


# HIV Reverse Transcriptase, with inhibitor Nevirapine (J. Ren et al. 2001, J.Mol.Bio. 312)



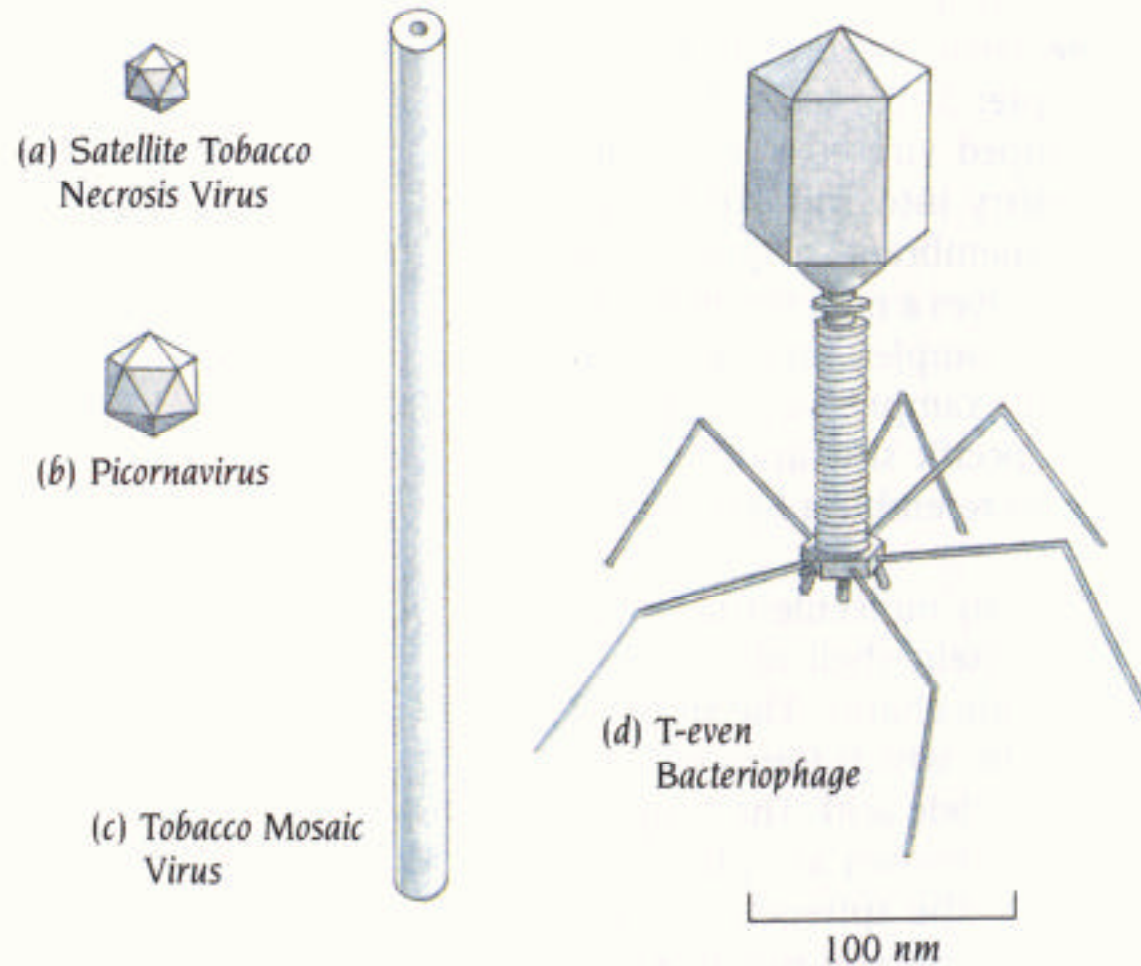


# Nevirapine alters the RNA binding site. RT resistance results from mutations of Cys181 or Tyr188

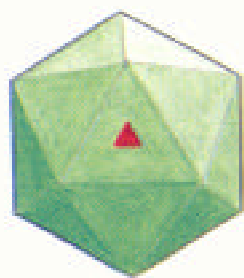
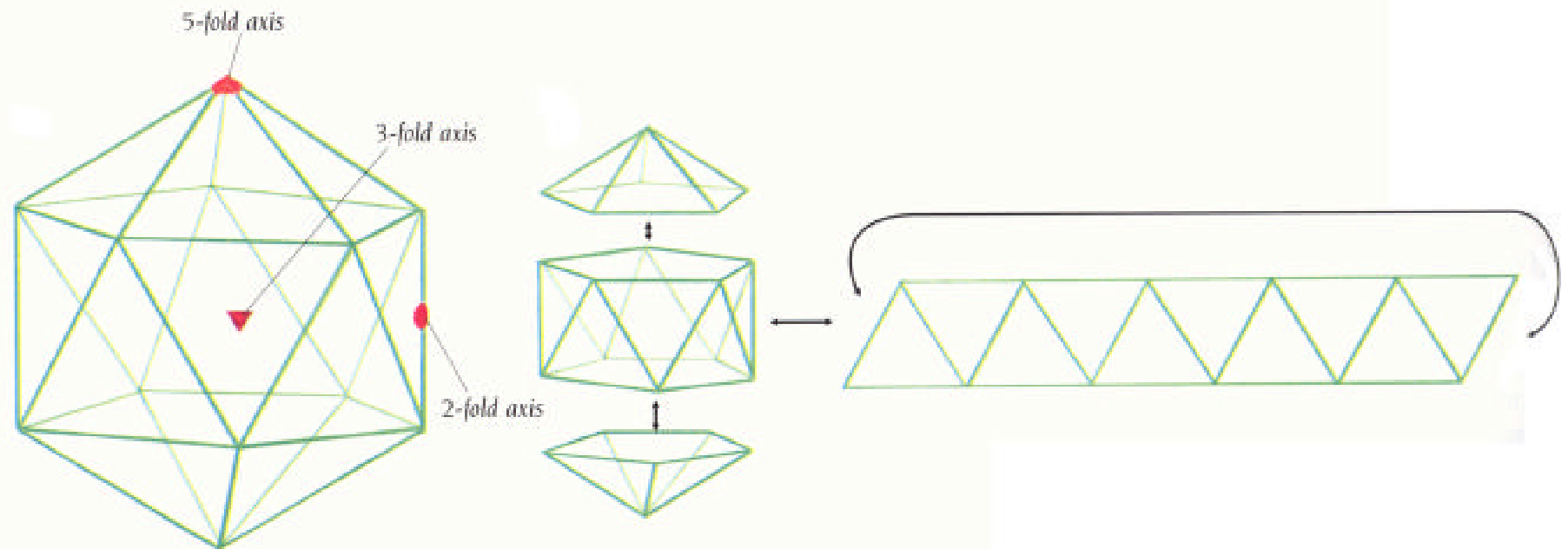




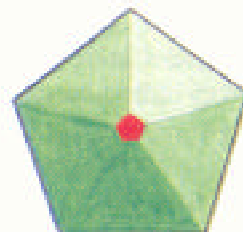
# Viruses



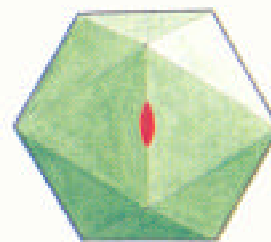




3-fold



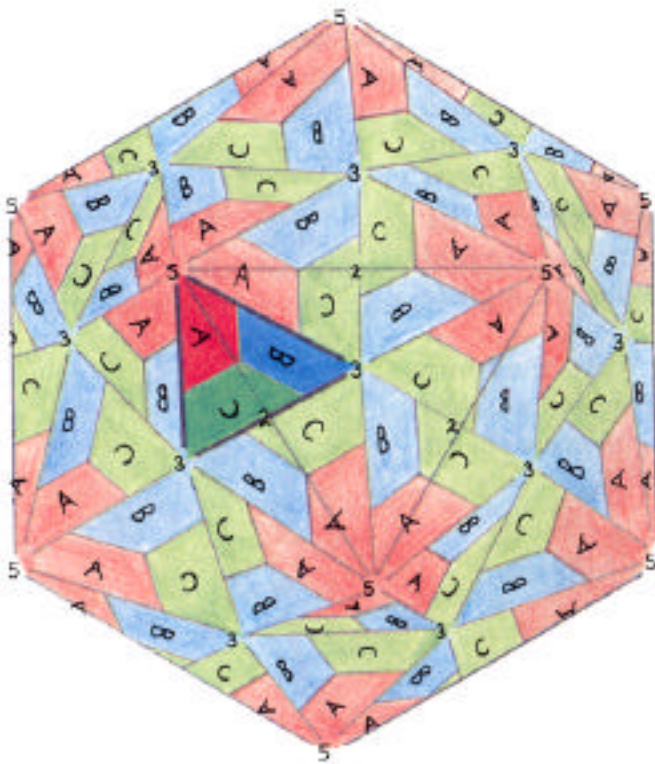
5-fold



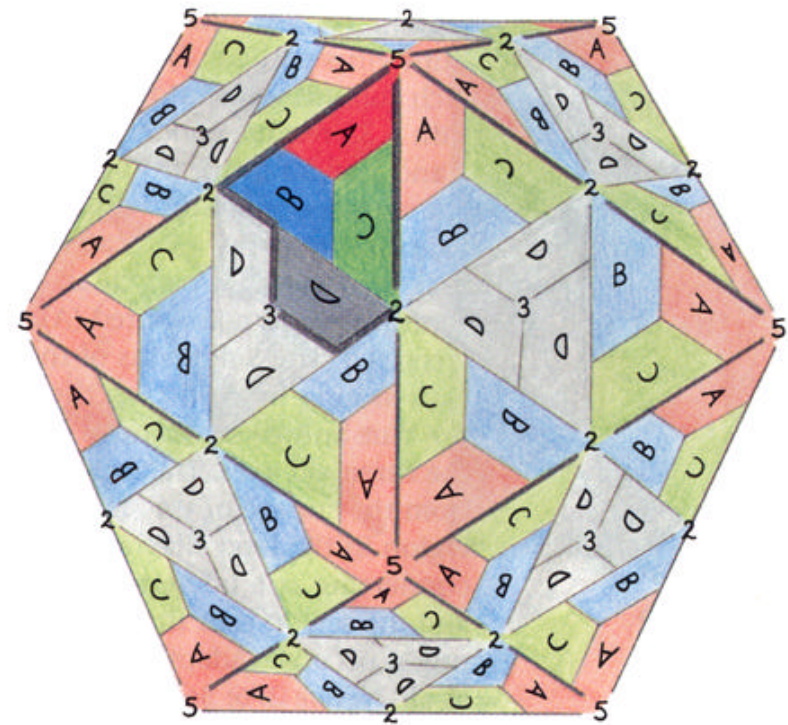
2-fold



Viruses with T-numbers  $>1$   
have identical coat proteins  
with *quasi-equivalent* shapes



**T3 symmetry**



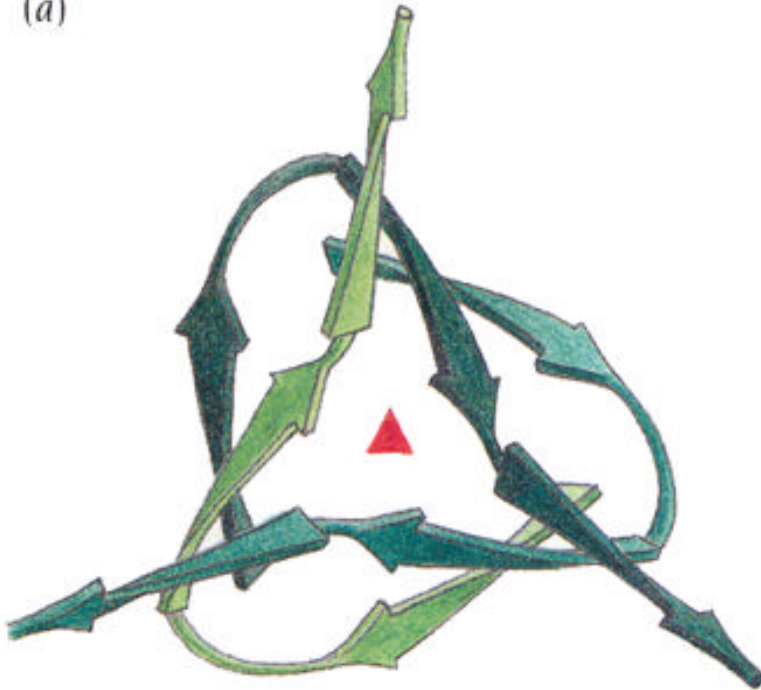
**T4 symmetry**



In the Tomato Bushy Stunt Virus,  
the C-termini tie knots (actually  
each is a tight 3-strand  $\beta$ -sheet)  
at each 3-fold vertex.



(a)

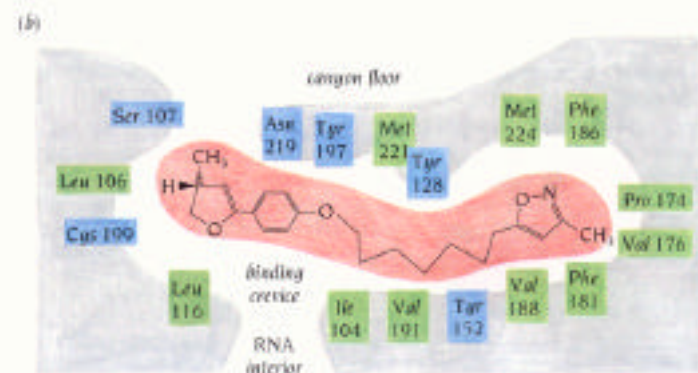
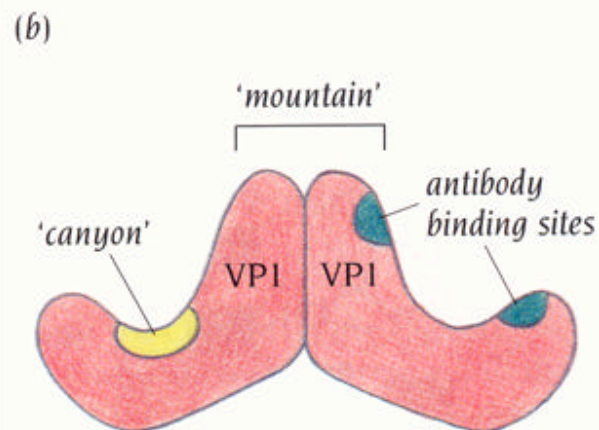
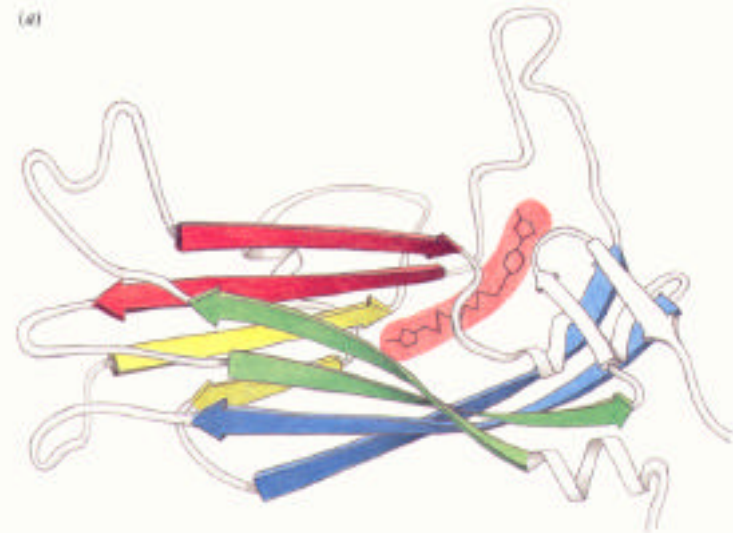
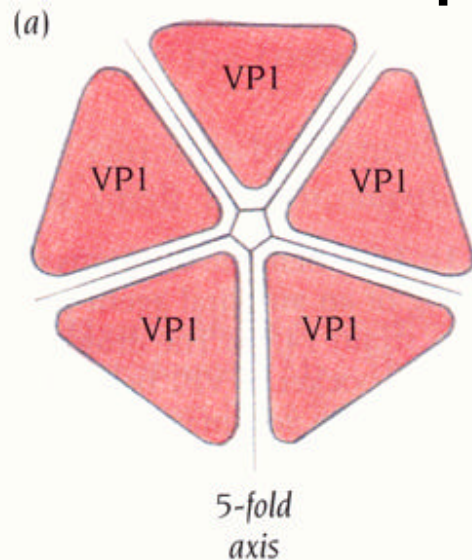


(b)





In Human Cold Virus, a “Canyon” surrounds the 5-fold. Antibodies cannot bind there, but ICAM-1 (the Rhinovirus receptor adhesin) does.







# Multiwire Detectors

- **Counting detector: Poisson statistics, no dark image**
  - **VERY efficient at low-to-medium count rates: excellent choice for rotating-anode x-ray source, or low-signal synchrotron applications**
  - **Can be made large, but not VERY large**
  - **Histogram image; electronic dead time for each count, but no “readout” lag**
  - **Not expensive**
- **Problem #1: pixels are big (0.2-1.0 mm), limiting spatial resolution**
- **Problem #2: limited speed/count rate**



# Imaging Phosphor Plates



- Integrating detector, but no dark image
  - Can be made VERY large (at expense of speed)
  - Modest spatial resolution (0.15 mm), but large size permits substantial resolving power
  - More expensive than multiwires
- Problem #1: slow readout; long dead time between image frames
  - Problem #2: dynamic range and DQE are limited
- Despite many attractive features, there is little prospect for further development of this technology



# Fiberoptically-coupled CCD Detectors



- Integrating detector; exhibits dark image
  - Spatial resolution better than IPs or MWs (FWHM 0.05 mm, vs 0.15 mm for IPs)
  - Very dense pixel rasters; can be made modular
  - Resolving power of multi-modular CCD system surpasses IPs
  - Fast readout (<1s) permits fine phi slicing
  - Higher efficiency than phosphor plates
- 
- Problem #1: Expensive to make very large
  - Problem#2: Limited dynamic range
  - Problem #3: Fast, but not fast enough
  - Problem #4: Image degraded by fibers (chickenwire, vignetting, blemishes, etc.)





# Amorphous silicon TFT Arrays

- Integrating detector; exhibits dark image
  - Can easily be made very large
  - Sharp spatial resolution: no “tails”
  - Current embodiment has low (12-bit) dynamic range; technology ***could*** have higher dynamic range.
  - DQE limited by very high read noise: engineering needed.
  - Can be made inexpensively
  - Reasonable readout speed (2-10 seconds)
- 
- **Problem#1: converting X-ray image to electronic image: needs a photoconducting converter, not a phosphor**
  - **Problem#2: very noisy medium**





# Lens-coupled CCD Detectors

- Uses one single CCD
  - Integrating detector; exhibits dark image
  - Can be made very large
  - Dynamic range and DQE equivalent to modular CCDs
  - Fast readout; short dead time between image frames, permits fine phi slicing
  - Easier to calibrate and correct than modular systems
  - Inexpensive
- **Problem#1: needs custom-designed, very-high numerical aperture lens**
  - **Problem#2: needs careful engineering to “get it right”**



# Pixel Array Detectors

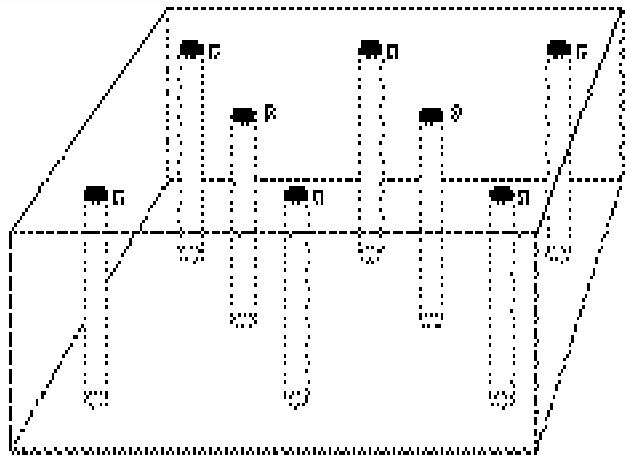


- Counting detector system: Poisson statistics, no dark image
- Efficient even at high count rates
- Sharp spatial resolution: no “tails”
- Large detectors must be modular
- Expensive to develop; may be inexpensive to produce

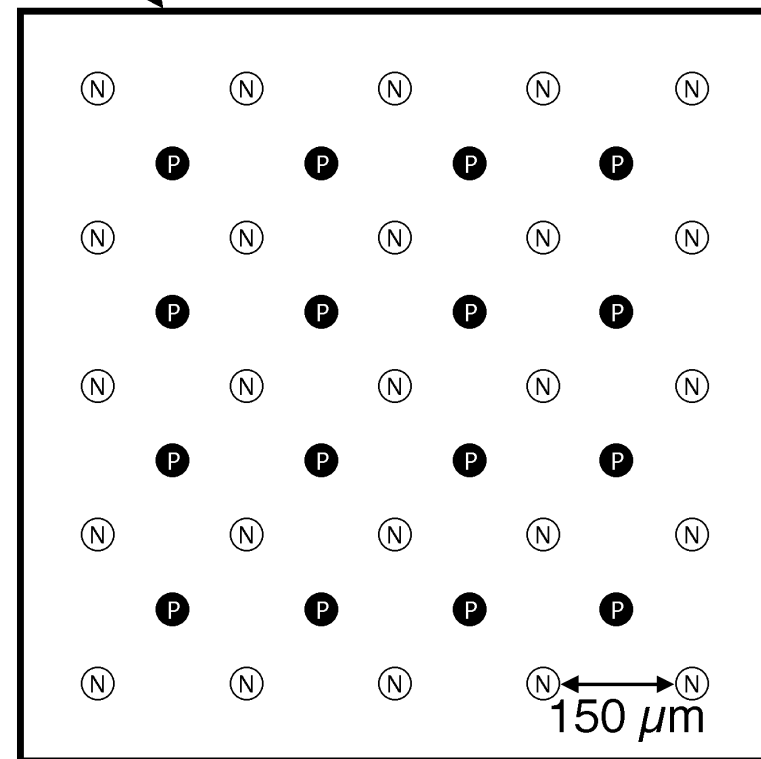


# Silicon "3DX" Concept

- Micromachined silicon detection wafer:



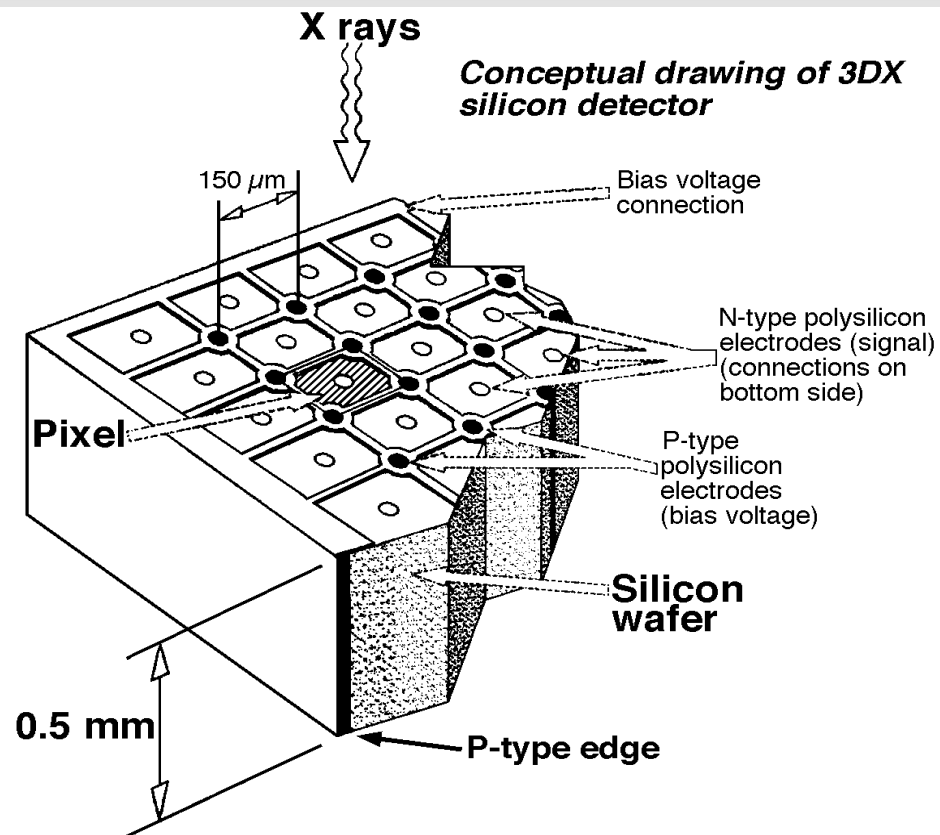
edge is p-type polycrystalline silicon ("poly")



edge same potential as p-type electrodes

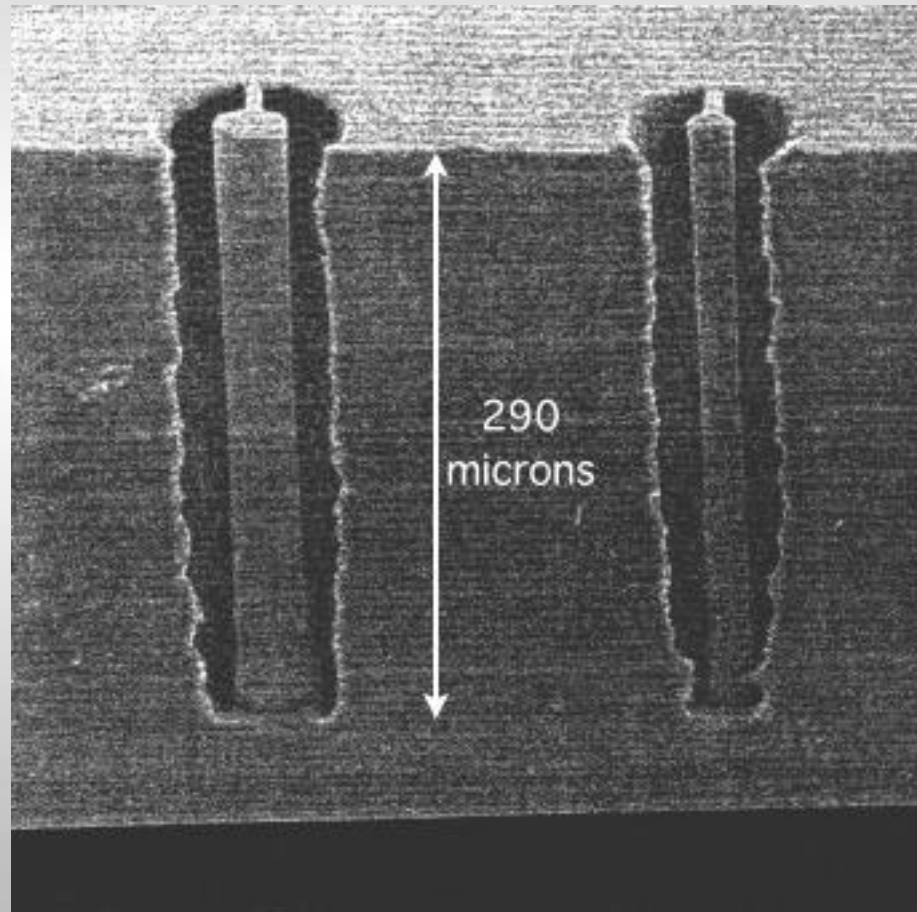


# Detector Wafer Processed into Pixels



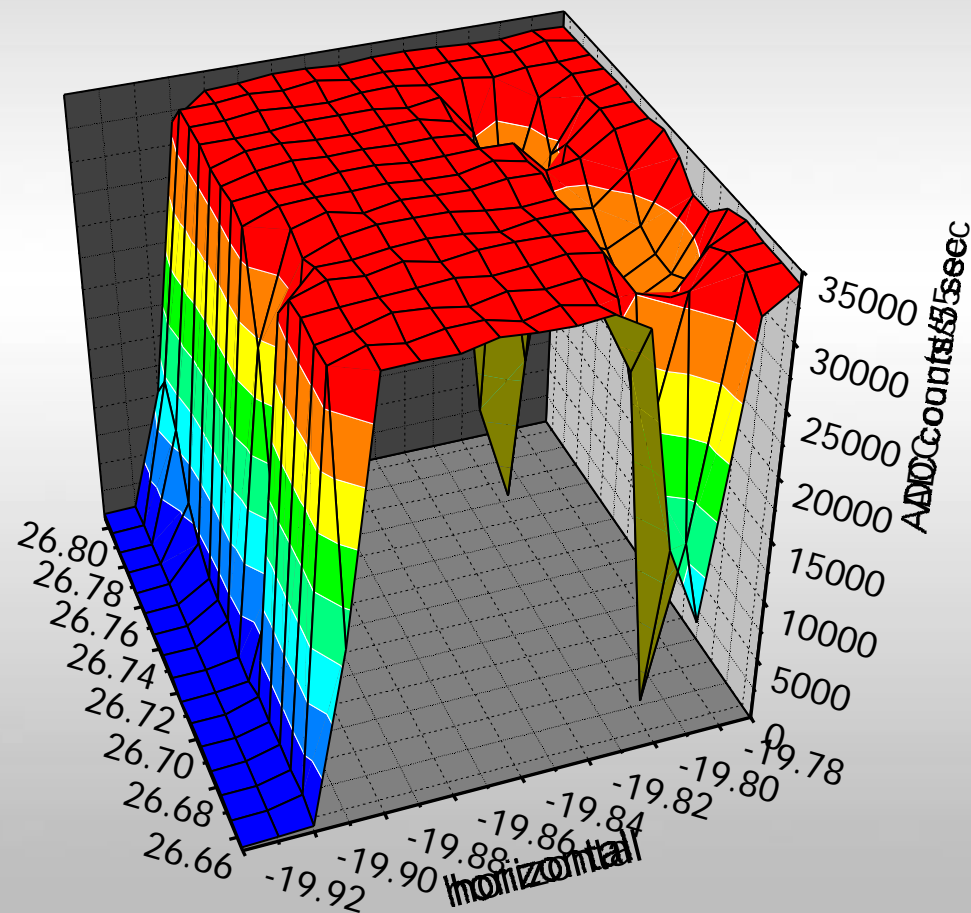


# Micromachining Silicon with High Aspect Ratios





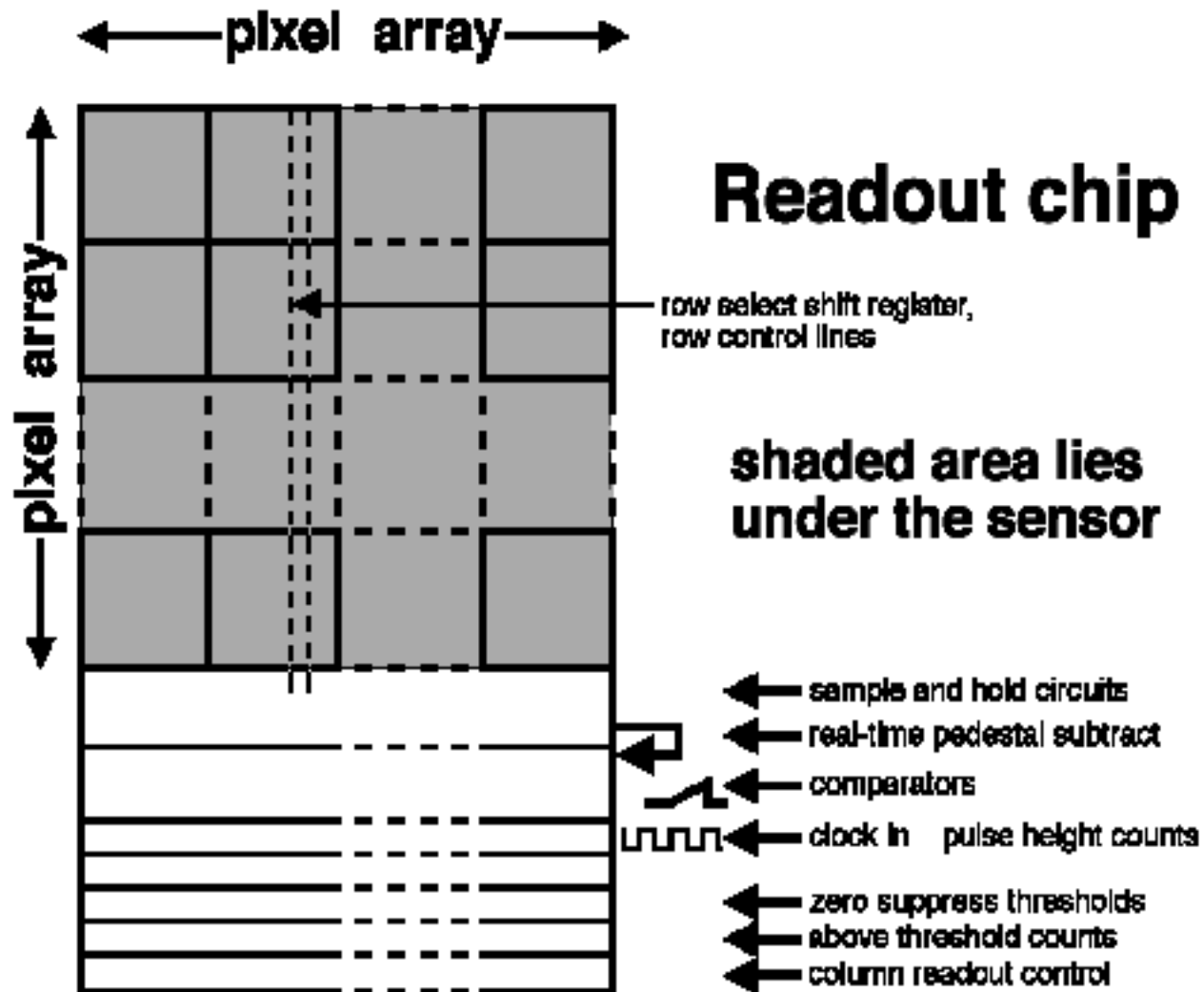
Excellent response uniformity  
(except at electrodes!)





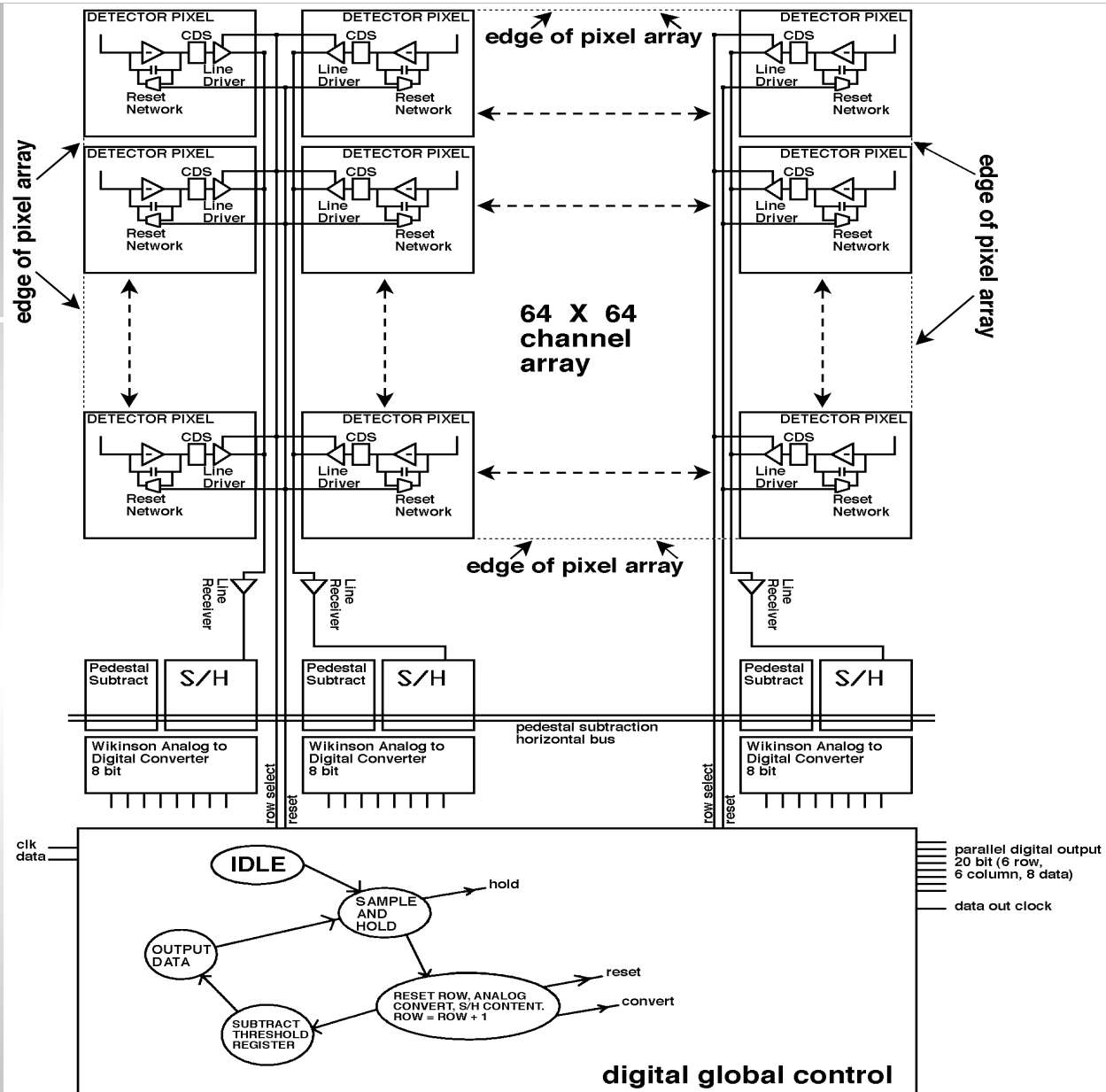
# Hybrid Technology: Separate Sensor & Readout Chips

“Deep sub-micron”  
CMOS circuit design  
for readout wafer





64 x 64 array  
150  $\mu\text{m}$  x 150  $\mu\text{m}$  pixels



S/H : Sample and Hold  
CDS: Correlated Double Sampling



# Concept for Tiling Large Areas

